Metabolomic Characterization of the Salt Stress Response in Streptomyces coelicolor

Kol, Stefan; Merlo, M. Elena; Scheltema, Richard A.; de Vries, Marcel; Vonk, Roel J.; Kikkert, Niels A.; Dijkhuizen, Lubbert; Breitling, Rainer; Takano, Eriko

Published in:
Applied and environmental microbiology

DOI:
10.1128/AEM.01992-09

Publication date:
2010

Supplementary Material

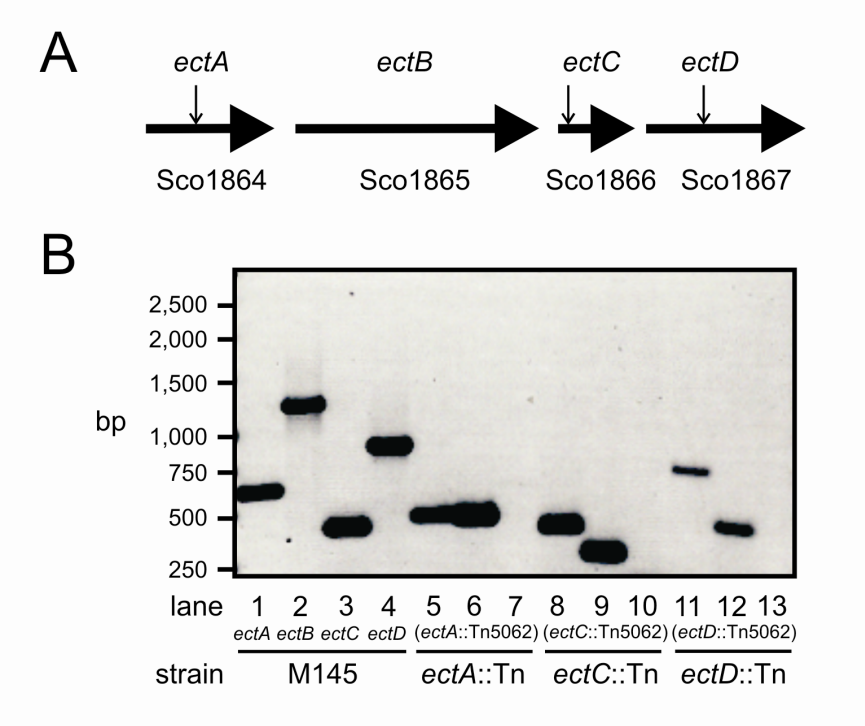


Figure A: Disruption of ectoine biosynthesis genes. (A) Genomic organization of the ectoine biosynthesis cluster in *S. coelicolor* with the location of the transposon insertion denoted by an arrow. (B) PCR using genomic DNA of M145 with primers annealing around the start and stop codons of *ectA*, *ectB*, *ectC* and *ectD* (lane 1–4, respectively). The same reaction was performed on genomic DNA of *ectA*::Tn, *ectC*::Tn and *ectD*::Tn using the start and stop codon primers (lane 7, 10 and 13), the start codon primer and the EZL1 primer (lane 5, 8 and 11) and the stop codon primer and the EZR1 primer (lane 6, 9 and 12).

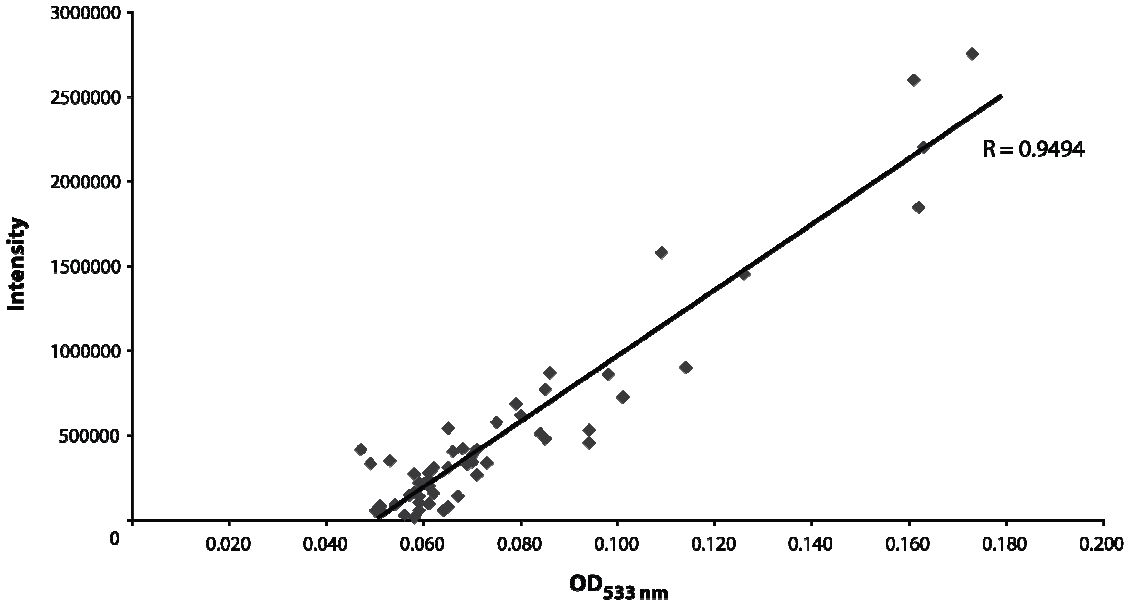


Figure B: Accuracy of the Orbitrap-LTQ quantification. Optical density levels were determined for the abundance of the antibiotic undecylprodigisins in the methanol cell extracts and compared to the mass spectrometric intensity levels of the corresponding masses (391.2623627: butylcycloheptylprodigiosin, metacycloprodiginine and methyl-cyclo-decyl-prodiginine; 393.2780128: undecylprodiginine) measured on the Orbitrap-LTQ. The high correlation between the two measures indicates that the quantification on the Orbitrap-LTQ is linear over a wide range.

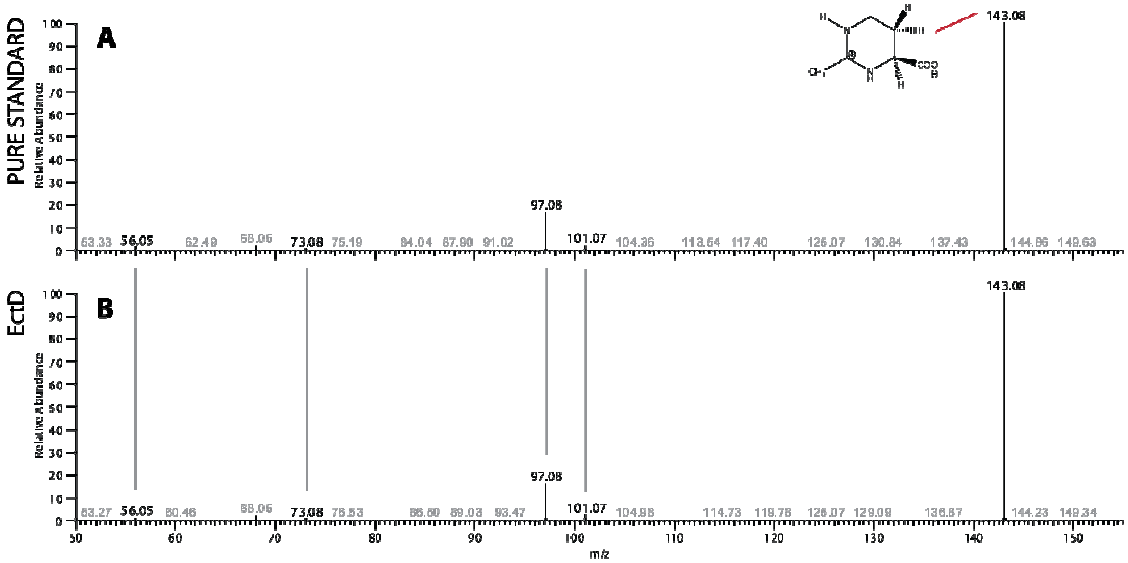


Figure C: MS/MS on synthesized ectoine (A) and the ectoine found in the EctD knockout during continuous salt stress (B). A peak at a retention time of 4 minute 56 seconds was observed in both samples corresponding to a mass of 142.0742276 amu. Four fragment masses were found to match between the ectoine in the pure standard and ectoine found in the *ectD* strain, resulting in a positive identification of ectoine in the *ectD* knockout.

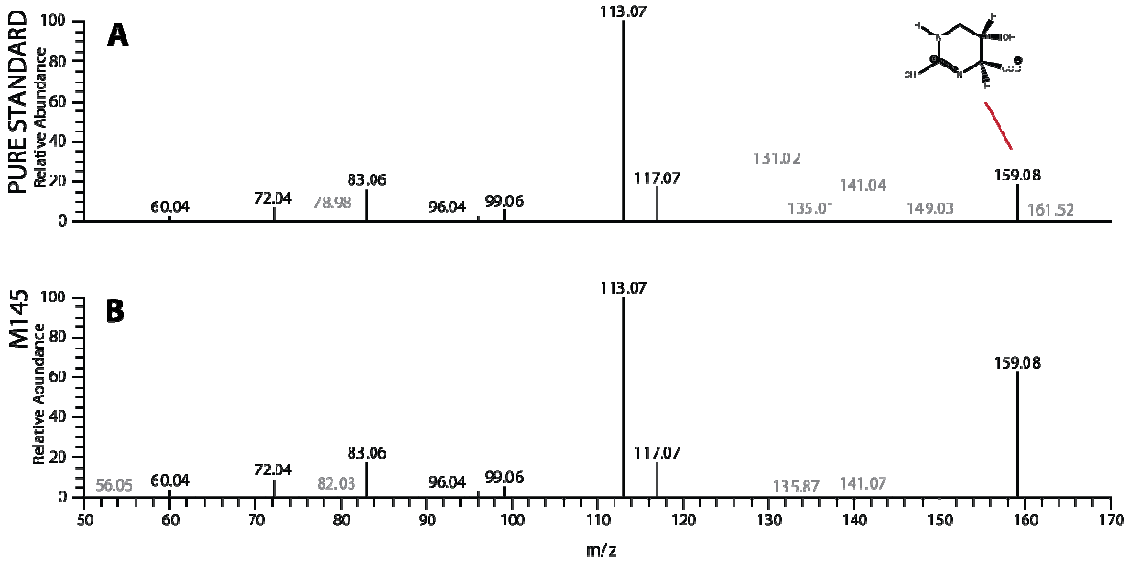
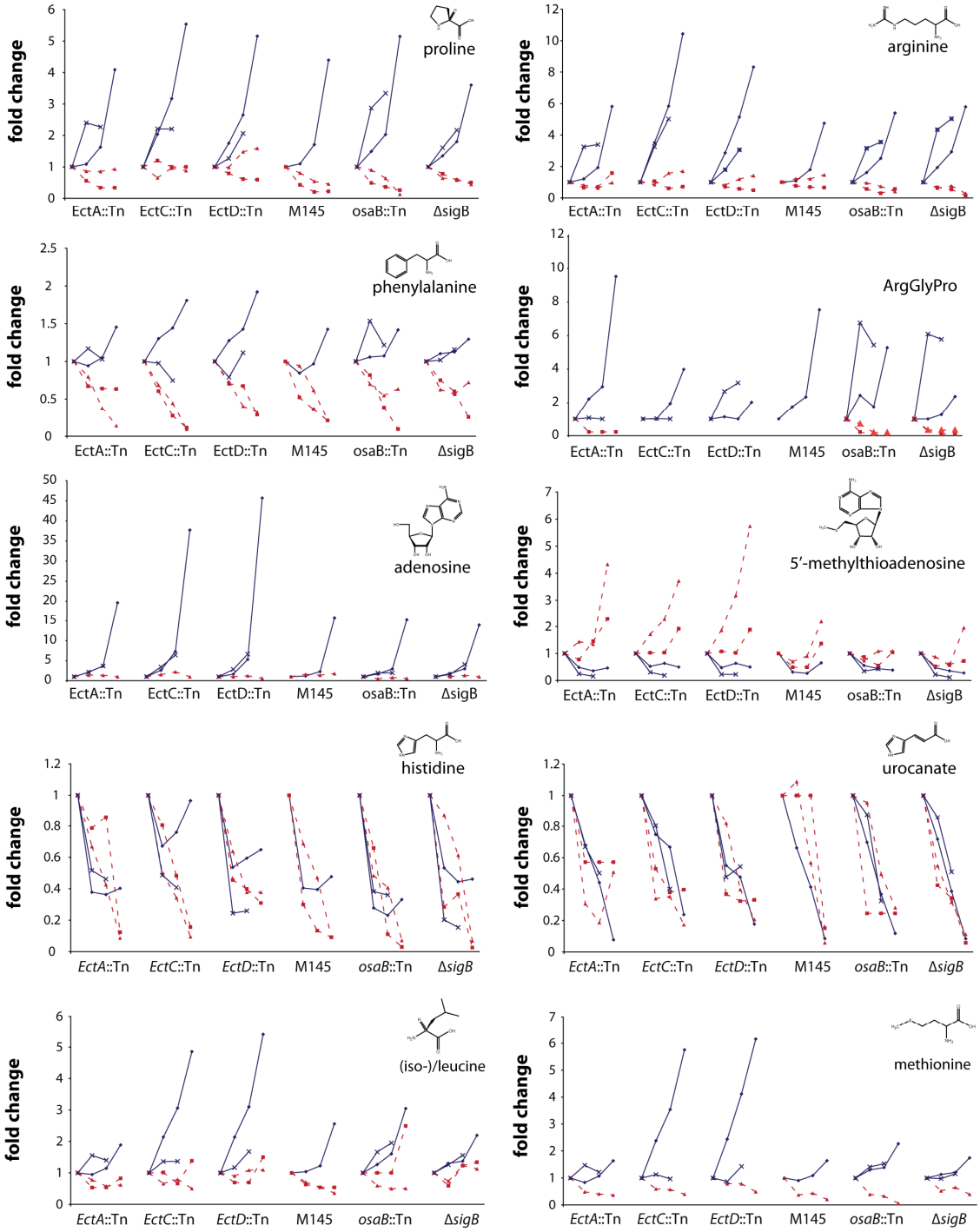


Figure D: MS/MS on synthesized hydroxyectoine (A) and the hydroxyectoine found in the parent strain during continuous salt stress (B). A peak at a retention time of 6 minute 22 seconds was observed in both samples corresponding to a mass of 158.0691422 amu. Seven fragment masses were found to match between the hydroxyectoine in the pure standard and hydroxyectoine found in the M145 strain, resulting in a positive identification of ectoine in the *ectD* knockout.


fold change
proline
arginine
phenylalanine
ArgGlyPro
adenosine
5′-methylthioadenosine
histidine
urocanate
(iso-)/leucine
methionine
EctA::Tn
EctC::Tn
EctD::Tn
M145
osaB::Tn
ΔsigB

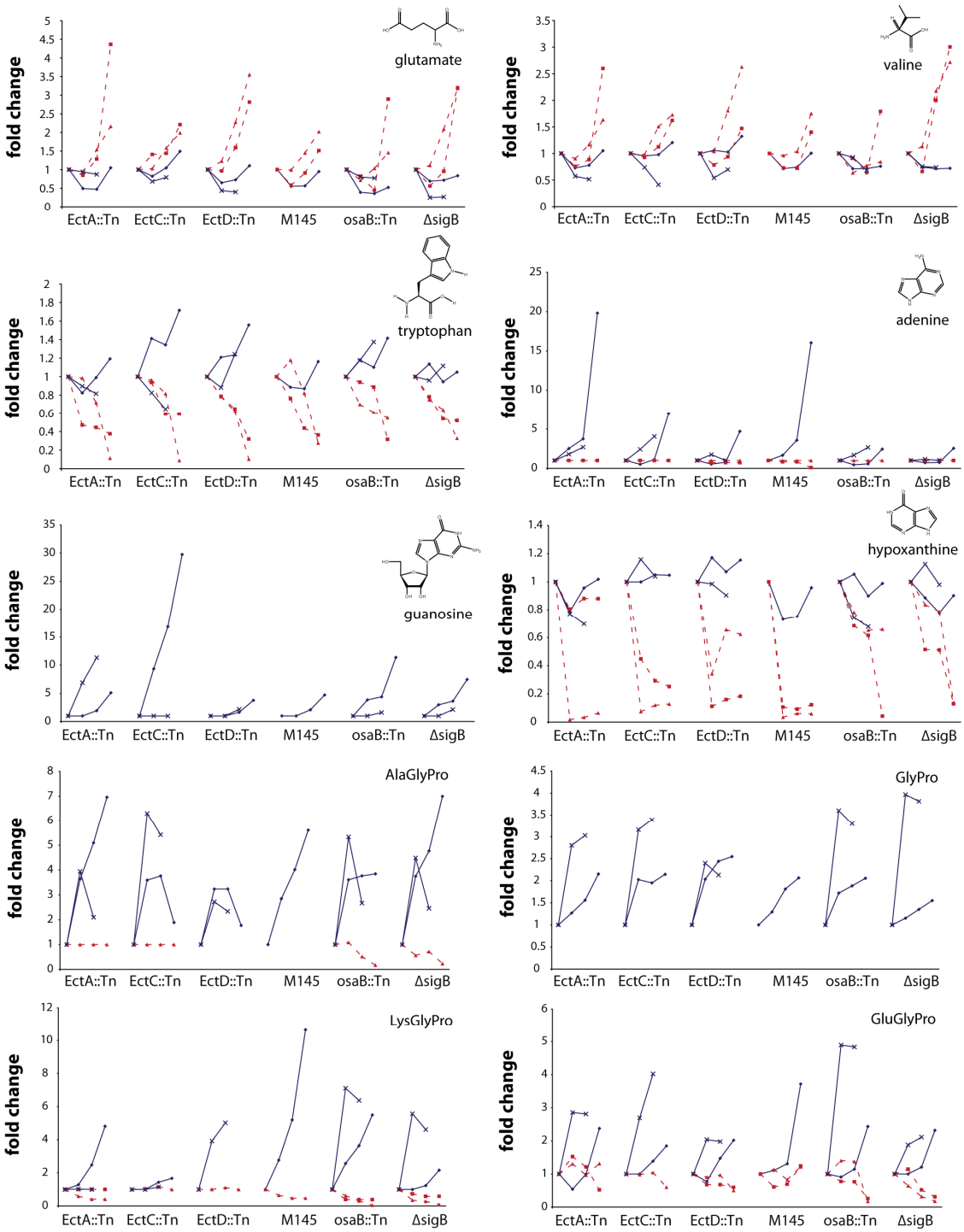


Figure E: Time-trends for reproducible, putatively identified stress responsive metabolites. The twenty putatively identified metabolites shown here have reproducible behavior in response to salt shock, as determined in two independent series of experiments (dashed red lines: control experiments without salt; solid blue lines: salt shock time courses with 3 and 4 time points). These plots illustrate the reproducibility

and biological variation in our experiments, even though slightly different time points and assay conditions were used for each experiment. The major osmoprotectant proline, as well as several other putatively identified amino acids shows an increase in response to the salt shock. The detected proline/glycine-containing di- and tripeptides also accumulate in response to the salt shock (ArgGlyPro is shown as a representative example), as do a few nucleotide-related compounds, including adenosine.

Table A: List of observed masses corresponding to peak groups and their putative identifications in the time series of salt-shocked *S. coelicolor* strains. The list is divided in 8 classes: amino acids, amino acid derivatives, nucleotide derivatives, vitamins and derivatives, LC-MS contaminants, di- and tri-peptides, diverse *S. coelicolor* and non-*S. coelicolor* compounds.

Amino acids :

Observed Mass	Formula	Calculated theoretical mass	Putative identification
89.04767847	$C_3H_7NO_2$	89.04732734	Alanine
115.0633285	$C_5H_9NO_2$	115.062862	Proline
117.0789786	$C_5H_{11}NO_2$	117.0785008	Valine
125.0146638	$C_2H_7NO_3S$	125.014144	Taurine
131.0946287	$C_6H_{13}NO_2$	131.0940858	Isoleucine
131.0946287	$C_6H_{13}NO_2$	131.0940858	Leucine
146.1055277	$C_6H_{14}N_2O_2$	146.1049136	Lysine
147.0531578	$C_5H_9NO_4$	147.0524593	Glutamate
149.0510493	$C_5H_{11}NO_2S$	149.050386	Methionine
155.0694765	$C_6H_9N_3O_2$	155.0687415	Histidine
165.0789786	$C_9H_{11}NO_2$	165.0781586	Phenylalanine
174.1116757	$C_6H_{14}N_4O_2$	174.1108765	Arginine
175.0956913	$C_6H_{13}N_3O_3$	175.0949079	Citrulline
181.0738932	$C_9H_{11}NO_3$	181.0730399	Tyrosine
188.1273258	$C_7H_{16}N_4O_2$	188.1264433	Homoarginine
204.0898776	$C_{11}H_{12}N_2O_2$	204.0889605	Tryptophan
146.0684584	$C_5H_{10}N_2O_3$	146.0694166	Glutamine

Amino acid derivatives:

Observed Mass	Formula	Calculated theoretical mass	Putative identification
87.06841392	C_4H_9NO	87.06807249	4-aminobutanal
129.0425931	$C_5H_7NO_3$	129.0420565	5-oxoproline
131.0582432	$C_5H_9NO_3$	131.0576974	Glutamate-γ-semialdehyde
138.0429274	$C_6H_6N_2O_2$	138.0423681	Urocanate
139.0745619	$C_6H_9N_3O$	139.0739442	Histidinal
145.1102787	$C_7H_{15}NO_2$	145.1096065	γ -butyrobetaine
161.1051934	$C_7H_{15}NO_3$	161.1044273	Carnitine
174.0640568	$C_6H_{10}N_2O_4$	174.0632077	N-formimino-glutamate
174.1004423	$C_7H_{14}N_2O_3$	174.0996361	N-acetylornithine
188.0797069	$C_7H_{12}N_2O_4$	188.0787774	N-acetylglutamine
218.1266571	$C_9H_{18}N_2O_4$	218.1255806	N2-(D-1-carboxyethyl)-lysine
229.0884974	$C_9H_{15}N_3O_2S$	229.0874781	Ergothioneine
240.1222404	$C_{10}H_{16}N_4O_3$	240.1209052	Anserine
268.0551484	$C_8H_{16}N_2O_4S_2$	268.0535821	Homocystine
297.0895601	$C_{11}H_{15}N_5O_3S$	297.0879508	5'-methylthioadenosine

Nucleotide derivatives:

Observed Mass	Formula	Calculated theoretical mass	Putative identification
73.06399724	$C_2H_7N_3$	73.06377018	Methylguanidine
111.0432618	$C_4H_5N_3O$	111.0428494	Cytosine
112.0272774	$C_4H_4N_2O_2$	112.0268192	Uracil
135.0544952	$C_5H_5N_5$	135.0539224	Adenine
136.0385108	$C_5H_4N_4O$	136.0379461	Hypoxanthine
151.0494098	$C_5H_5N_5O$	151.0487018	Guanine
152.0334254	$C_5H_4N_4O_2$	152.0327149	Xanthine
243.0855205	$C_9H_{13}N_3O_5$	243.0842464	Cytidine
267.0967539	$C_{10}H_{13}N_5O_4$	267.0953564	Adenosine/deoxyguanosine
268.0807695	$C_{10}H_{12}N_4O_5$	268.0793525	Inosine
283.0916686	$C_{10}H_{13}N_5O_5$	283.0902003	Guanosine

Vitamins and derivatives:

Observed Mass	Formula	Calculated theoretical mass	Putative identification
123.0320284	$C_6H_5NO_2$	123.0315361	Nicotinate
205.1314081	$C_9H_{19}NO_4$	205.1303806	Pantothenol
244.0881631	$C_{10}H_{16}N_2O_3S$	244.0869737	biotin
278.1266571	$C_{14}H_{18}N_2O_4$	278.1252443	α-ribazole

LC-MS Contaminants:

Observed Mass	Formula	Calculated theoretical mass	Putative identification
71.03711379	C_3H_5NO	71.03690097	Acrylamide
58.0530982	CH_3CN	58.05299784	Acetonitrile
59.07349929	C_3H_9N	59.07338579	Trimethylamine (TMA) or isopropylamine
73.05276385	(CH3CN)(CH3OH)	73.05252293	ESI solvent Acetonitrile/methanol
99.06841392	C_5H_9NO	99.06803865	N-Methyl-2-pyrrolidone (NMP)
101.1204495	$C_6H_{15}N$	101.1200791	Triethylamine (TEA)
114.079313	(CH3CN)(C3H7NO)	114.0789002	Solvent Acetonitrile/dimethylformamide
121.0738932	$C_4H_{11}NO_3$	121.0734126	Tris-hydroxymethyl-aminomethane (TRIS) buffer
129.1517496	$C_8H_{19}N$	129.151169	Diisopropylethylamine (DIPEA)
148.016044	$C_8H_4O_3$	148.0153541	Pthalic anhydride (phthalate esters fragment ion)
149.1051934	$C_6H_{15}NO_3$	149.1045214	Triethanolamine
150.0892089	$(C_2H_4O)_3H_2O$	150.0884136	Polyethylene glycol (PEG)
181.1830497	$C_{12}H_{23}N$	181.1821482	Dicyclohexylamine (DCHA)
185.2143499	$C_{12}H_{27}N$	185.2134235	Tributylamine (TBA)
194.1154237	$(C_2H_4O)_4H_2O$	194.1144716	Polyethylene glycol (PEG)
250.1780239	$(C_3H_6O)_4H_2O$	250.1766171	Polypropylene glycol (PPG)
337.334465	$C_{22}H_{43}NO$	337.3326626	Erucamide

Di- and tri-peptides:

Observed Mass	Formula	Calculated theoretical mass	Putative identification
172.0847923	$C_7H_{12}N_2O_3$	172.0839859	GlyPro
188.1160924	$C_8H_{16}N_2O_3$	188.1152413	GlyLeu
202.1317425	$C_9H_{18}N_2O_3$	202.1307894	AlaLeu/AlaIle
217.1426415	$C_9H_{19}N_3O_3$	217.1415924	AlaLys
218.0902716	$C_8H_{14}N_2O_5$	218.0891704	AlaGlu
222.1004423	$C_{11}H_{14}N_2O_3$	222.0993432	GlyPhe
229.106256	$C_9H_{15}N_3O_4$	229.1051152	AsnPro/GlyGlyPro
230.1630426	$C_{11}H_{22}N_2O_3$	230.1618796	IleVal/LeuVal
231.1331394	$C_8H_{17}N_5O_3$	231.1319816	ArgGly
243.1219061	$C_{10}H_{17}N_3O_4$	243.1206321	AlaGlyPro/GlnPro
243.1219061	$C_{10}H_{17}N_3O_4$	243.1206383	GlnPro
244.1786926	C12H24N2O3	244.1773854	IleIle/LeuLeu
245.1487895	C9H19N5O3	245.1475177	AlaArg
245.1739416	$C_{11}H_{23}N_3O_3$	245.1726794	LysVal
246.1215717	$C_{10}H_{18}N_2O_5$	246.1203201	GluVal/AspLeu/AspIle
259.1168207	$C_{10}H_{17}N_3O_5$	259.1154725	GluPro
259.1532062	$C_{11}H_{21}N_3O_4$	259.1519151	AlaGlyLeu/AlaGlyIle/GlnIle/GlnLeu/AlaAlaVal
260.1372218	$C_{11}H_{20}N_2O_5$	260.1359108	GluIle/GluLeu
274.1641052	$C_{11}H_{22}N_4O_4$	274.1626526	GlnLys
285.1324707	$C_{12}H_{19}N_3O_5$	285.1309912	GlyPro(OH-)Pro
287.1845063	$C_{13}H_{25}N_3O_4$	287.1829871	AlaValVal/GluIleVal/GluLeuVal
300.1797553	$C_{13}H_{24}N_4O_4$	300.1782043	GlyLysPro
301.1273854	$C_{12}H_{19}N_3O_6$	301.1258452	AlaAspPro/GluGlyPro
301.2001564	$C_{14}H_{27}N_3O_4$	301.1984929	AlaIleVal/AlaLeuVal/GlyIleIle/GlyLeuLeu
318.117549	$C_{11}H_{18}N_4O_7$	318.1159027	AsnGluGly/AspGlnGly
318.1328051	$C_{15}H_{18}N_4O_4$	318.1313075	GlyGlyTrp/AsnTrp/HisTyr
328.1859033	$C_{13}H_{24}N_6O_4$	328.1842734	ArgGlyPro
342.19032	$C_{15}H_{26}N_4O_5$	342.1886168	AsnIlePro/AsnLeuPro/GlnProVal
356.20597	$C_{16}H_{28}N_4O_5$	356.2041008	GlnIlePro/GlnLeuPro
356.2423555	$C_{17}H_{32}N_4O_4$	356.2406342	LeuLysPro/LysIlePro
371.2168691	$C_{16}H_{29}N_5O_5$	371.2148124	GlnLysPro
372.2372702	$C_{17}H_{32}N_4O_5$	372.2354454	GlnIleLeu/GlnIleIle/GlnLeuLeu

Diverse *Streptomyces coelicolor* compounds :

Observed Mass	Formula	Calculated theoretical mass	Putative identification
391.2623627	$C_{25}H_{33}N_3O$	391.2604	Meta-cyclo-prodiginine
393.2780128	$C_{25}H_{35}N_3O$	393.27603	Undecylprodigine

Diverse non-*Streptomyces coelicolor* compounds :

Observed Mass	Formula	Calculated theoretical mass	Putative identification
59.07349929	C_3H_9N	59.073366	Trimethylamine
69.05784923	C_4H_7N	69.057655	Pyrroline
71.07349929	C_4H_9N	71.073288	Pyrrolidine
97.9768951	H_3O_4P	97.976483	Phosphate
141.0789786	$C_7H_{11}NO_2$	141.0784	Guvacoline
142.0742276	$C_6H_{10}N_2O_2$	142.07363	ectoine
143.0946287	$C_7H_{13}NO_2$	143.09402	Stachydrine
144.0898776	$C_6H_{12}N_2O_2$	144.08925	
147.0895433	$C_6H_{13}NO_3$	147.08881	Fagomine
148.0524295	$C_9H_8O_2$	148.05178	coumaraldehyde
157.1102787	$C_8H_{15}NO_2$	157.10954	Homostachydrine
176.0507149	$C_7H_{12}O_3S$	176.04962	2-Oxo-6-methylthiohexanoic acid
190.066365	$C_8H_{14}O_3S$	190.06523	2-Oxo-7-methylthioheptanoic acid
194.0803756	$C_8H_{10}N_4O_2$	194.07947	Caffeine
204.0721191	$C_{11}H_{12}N_2S$	204.07101	Levamisole
207.1623143	$C_{13}H_{21}NO$	207.16128	Luciduline
223.1208434	$C_{12}H_{17}NO_3$	223.11983	Cerulenin
228.111007	$C_{10}H_{16}N_2O_4$	228.10989	(S)-ATPA
240.1473925	$C_{12}H_{20}N_2O_3$	240.14617	Slaframine
247.1936144	$C_{16}H_{25}NO$	247.19229	Lycopodine
315.204573	$C_{16}H_{29}NO_5$	315.20288	Butoctamide hydrogen succinate
350.1392331	$C_{21}H_{20}NO_4$	350.13802	Fagaronine
444.2260368	$(C_{20}H_{26}N_2)(C_4H_6O_6)$	444.22384	Dimetacrine tartrate

Table B: Oligonucleotides used for PCR in Supplementary Material Figure A.b to confirm the ectoine disruption mutant construct.

Oligonucleotides	Sequence
Fw EctA	5'-GCTTACGCACCCGTCAAATC-3'
Rv EctA	5'-GGTCCGATGCGGTACAGGAC-3'
Fw EctC	5'-CGCAAAACCGTGATCGTCCG-3'
Rv EctC	5'-GGTGACTCACACCTCCTCGG-3'
Fw EctD	5'-CGAACGTCACTGATCTCTATCC-3'
Rv EctD	5'-CGAGTCACTTCACCGGCG-3'

Table C: Masses found by unsupervised hierarchical clustering. Cluster 1 contains all the masses found with an increasing trend over time and cluster 4 all the masses found with a decreasing trend over time. Putative identifications are listed.

Cluster 1		**Cluster 4**	
Mass	**Putative identification**	**Mass**	**Putative identification**
71.073288		337.332663	erucamide
85.088820	leucine	103.099329	trimethyalamine
101.083698		138.042368	urocanate
112.026819	uracil	158.039182	
115.062862	proline	129.151169	DIPEA
118.995386		190.065213	
119.073003	phenylalanine	315.202883	butoctamide hydrogen succinate
133.010953		113.052327	
146.104914	lysine	237.207996	
148.984849		255.182082	
149.005767		297.087951	5'-methylthioadenosine
149.050386	methionine	312.264846	
151.048702	guanine	220.177634	
164.958671		122.047532	
171.197908		271.176922	
172.083986	ProGly	161.989719	
174.099636	N-α-acetylornithine	232.104773	
174.110877	arginine	257.197710	
188.115241	GlyLeu	229.087478	ergothioneine
189.984498			
204.073662			
217.141592	AlaLys		
218.125581	carboxyethyl lysine		
243.084246	cytidine		
243.120632	AlaProGly | GlnPro		
245.147518	AlaArg		
247.192286	lycopodine		
259.115473	linatine		
283.090200	guanosine		
285.130991	glycylprolylhydroxyproline		
300.178204	LysGlyPro		
325.369097	hypoxanthine		
328.184273	ProArgGly		